En Chem, Inc.

Quality Assurance Document

SET No: 1

Standard Operating Procedure

TITLE: Gel Permeation Chromatography

DEPARTMENT: Semivolatile Organic Extractions

REFERENCES: USEPA Contract Laboratory Program
Statement of Work for Organics Analysis
Document Number OLM01.0
Including Revision OLM01.8 (December 1990)

Test Methods for Evaluating Solid Waste
SW 846 method 3640A (3rd Ed., Rev. 1, July 1992)

Operation Manual for GPC Model 1002B
Analytical Bio-Chemistry Laboratories, Inc.
January 1990

PROCEDURE SUMMARY:

Gel permeation chromatography (GPC) is a size exclusion cleanup procedure using organic solvents and hydrophobic gels in the separation of synthetic macromolecules. GPC is required for all soil/sediment samples, regardless of concentration level, for the elimination of lipids, polymers, copolymers, proteins, natural resins, cellular components, viruses, steroids, and dispersed high-molecular weight compounds from the sample extract. Normally, this method is most efficient for removing high boiling materials that condense in the injection port area of a gas chromatograph (GC) or on the front of a GC column.

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APPARATUS AND MATERIALS:

Gel Permeation Chromatography System:

Chromatographic Column:	700 mm X 25 mm i.d. glass column. Column flow is upward. A three-way valve is used so the column exit flow can be shunted either to the UV flow-through cell or to the GPC collection device.
Bio Beads:	200-400 mesh, S-X3, Bio-Rad Chemical Division. The quality of bio beads may vary from lot to lot because of excessive fines in some lots.
Ultraviolet Detector:	Fixed wavelength (254 nm) with a semi-prep flow-through cell, and a strip chart recorder.
Syringe:	20 mL with Luerlock fitting, disposable, Becton Dickinson, Baxter Scientific.
Syringe Filter Assembly:	Millex-SR, 25mm, 0.5 micron filter unit. Millipore Products Division.

Reagents:

GPC Calibration Solution: GPC Calibration Solution, CLP-027, ACCU Standard, Inc. Prepare a calibration solution in methylene chloride containing the following analytes (in elution order) either by dilution of the above or the equivalent.

Compound	mg/mL
Corn Oil	25.0
Bis(2 ethylhexyl)phthalate	1.0
Methoxychlor	0.2
Perylene	0.02
Sulfur	0.08

Store the calibration solution in an amber glass vial with a teflon-lined screw cap. Store at 4 degrees centigrade protected from light. Replace the calibration solution every 6 months, or more frequently if necessary.

COLUMN PREPARATION:

Weigh out 70 gm of bio beads. Transfer them to a 2-L separatory funnel. Add approximately 500 mL of methylene chloride. Swirl the funnel to ensure the wetting of all beads. Allow the beads to swell for a minimum of two hours. Maintain enough solvent to cover the beads sufficiently at all times.

Turn the column upside down from its normal position, and remove the inlet bed support plunger (the inlet plunger is longer than the outlet plunger). Position and tighten the outlet bed support plunger as near the end as possible, but no closer than 5 cm (measured from the gel packing to the collar).

Raise the end of the outlet tube to keep the solvent in the GPC column, or close the column outlet stopcock. Place a small amount of solvent in the column to minimize the formation of air bubbles at the base of poured column packing.

Swirl the bead/solvent slurry to get a homogeneous mixture. Drain the excess methylene chloride directly into a waste beaker, and then start draining the slurry into the column by placing the separatory funnel tip against the column wall to minimize bubble formation. Swirl the separatory funnel occasionally to keep the slurry homogeneous and drain enough to fill the column. Place the tubing from the column outlet into a waste beaker below the column, open the stopcock, and allow excess solvent to drain. Raise the tube to stop the flow, and close the stopcock when the top of the gel begins to look dry. Add additional methylene chloride to just rewet the gel.

Wipe any remaining beads and solvent from the inner walls of the top of the column with a laboratory tissue. Loosen the seal slightly on the other plunger assembly (long plunger), and insert it into the column. Make the seal just tight enough so that any beads on the glass surface will be pushed forward, but loose enough so that the plunger can be pushed forward.

CAUTION: Do not tighten the seal if beads are between the seal and the glass surface because this can damage the seal and cause leakage.

Compress the column as much as possible without applying excessive force. Loosen the seal, and gradually pull out the plunger. Rinse and wipe off the plunger. Slurry any remaining beads, and transfer them into the column. Repeat the step in the previous paragraph, and reinsert the plunger. If the plunger can not be inserted and pushed in without allowing beads to escape around the seal, continue compression of the beads without tightening the seal, and loosen and remove the plunger as described. Repeat this procedure until the plunger is inserted successfully.

Push the plunger until it meets the gel, then compress the column bed about 4 cm.

Connect the column inlet to the solvent reservoir (reservoir should be placed higher than the top of the column), and place the column outlet tube in a waste container. Placing the restrictor in the outlet tube will force air out of the column more quickly. A restrictor can be fabricated from a piece of capillary stainless steel tubing of 1/16" o.d. X 10/1000" i.d. X 2". Pump methylene chloride through the column at a rate of 5 mL/min for one hour.

After washing the column for at least one hour, connect the column outlet tube, without the restrictor, to the inlet side of the UV detector. A restrictor (of the size in the previous paragraph), in the outlet tube from the UV detector will prevent bubble formation, which causes a noisy UV baseline, but will not affect flow rate. After pumping methylene chloride through the column for an additional 1-2 hours, adjust the inlet bed support plunger until approximately 6-10 psi back pressure is achieved. Push the plunger in to increase pressure, or slowly pull outward to reduce pressure.

When the GPC column is not to be used for several days, connect the column outlet line to the column inlet to prevent column drying and/or channeling. This can also be accomplished by closing the 3-port and 2-port valves in the solvent lines. If channeling occurs, the gel must be removed form the column, reswelled, and repoured as described above. If drying occurs, methylene chloride should be pumped through the column until the observed column pressure is constant and the column appears wet. Always recalibrate after column drying has occurred to verify retention volumes have not changed.

FILTRATION OF SOLUTIONS AND SAMPLES:

Particles greater than 5 microns may scratch the valve, which may result in a system leak and cross contamination of sample extracts in the sample loops. Filter the extract through a 5 micron filter by attaching the filter to the 20 mL syringe, transferring the extract into the syringe, and pushing the extract through the filter into the threaded glass-tube. Care should be exercised to avoid solvent contact with rubber syringe plunger.

OPERATION OF THE GPC SYSTEM:

Instrument Operation:

Operation of all instrument functions (ie. turning on solvent pump, setting sample load time, and sample analysis programming) are carried out through the use of the key pad.

The INDEX key is used to step through the startup procedure. Pressing the STANDBY key at any point will cause the process to stop.

Calibration of the GPC Column:

The GPC System must be calibrated weekly according to the following procedure:

Checking Solvent Flow Rate:

Depress INDEX until "CHECK SOLVENT FLOW RATE" is displayed. The solvent pump will start and column pressure will rise to operational level of 6-10 psi. Verify the flow rate by collecting column eluate in a 50 mL volumetric flask. Record the time required to attain 50 mL. Calculate the flow rate, which should be 4.5-5.5 mL/min. Record the column pressure, which should be 6-10 psi. Record room temperature. Changes in pressure, solvent flow rate, and temperature conditions can affect analyte retention times and must be monitored.

Initial Calibration

Using the 20 mL syringe and filter assembly, filter the calibration solution. The ABC automated system requires a minimum of 8 mL of calibration solution. Dilute 5 mL of calibration mix to 10 mL with methylene chloride. The filtered calibration mix is then loaded at an instrument sample port. Switch the valve so that GPC flow is through the UV-flow-through cell.

Recommended instrument settings are:

- Detector
  - Range (AUFS) = 0.5
  - Rise Time (sec) = 1.0

- Recorder
  - Millivolt (mv) Setting
  - Input = 10 mv
  - Chart Speed = 20 cm/Hr
  - Set zero knob to record

Activate the system, and obtain a UV trace showing a discrete peak for each component. The elution order is: corn oil, bis(2-ethylhexyl)phthalate, methoxychlor, perylene, and sulfur (refer to figure 1). Adjust the detector and/or recorder sensitivity to produce a UV trace that meets the following requirements:

- Peaks must be observed and should be symmetrical for all compounds in the calibration solution.

- Corn oil and phthalate peaks must exhibit > 85% resolution.

- Phthalate and methoxychlor peaks must exhibit > 85% resolution.

- Methoxychlor and perylene peaks must exhibit > 85% resolution.

- Perylene and sulfur peaks must not be saturated and must exhibit > 90% baseline resolution.

Calibration Check

Using the information from the UV trace, establish appropriate collect and dump time periods to ensure collection of all targets analytes. Initiate column eluate collection just before bis(2-ethylhexyl)phthalate and after the elution of the corn oil for semi-volatile samples. Initiate column eluate collection just before methoxychlor and after the elution of bis(2-ethylhexyl)phthalate for pesticide samples. Stop eluate collection after the elution of perylene and before the elution of sulfur for both semi-volatile and pesticide samples. Use a wash time of 15 minutes for both semi-volatile and pesticide samples. Refer to Instrument Programming for specific instructions on setting instrument parameters for calibration verification.

Analyze three GPC calibration verification solutions, which consist of the following:

- 1000 uL base-neutral/acid surrogate @ 100 ppm (B/N) and 150 ppm (acids) into a final volume of 10 mL methylene chloride.

- 1000 uL pesticide matrix spike solution (1.0 - 2.0 µg/mL) into a final volume of 10 mL methylene chloride.

- 1000 uL aroclor 1016/aroclor 1260 solution (2.0 µg/mL each) into a final volume of 10 mL methylene chloride.

Perform all dilutions in volumetric flasks.

After processing, refer to "FINAL CONCENTRATION OF EXTRACT" section to concentrate the semi-volatile blank.

Concentrate the pesticide and Aroclor verification solutions to 1.0 mL by attaching a three-ball Snyder column to the top of the Kuderna Danish flask and placing the flask on the hot (80-90 degrees C) water bath. Gently swirl the flask until boiling occurs. Concentrate to approximately 5 mL. Remove the flask from the water bath. Allow to drain and cool for 15 minutes. Remove the three-ball Snyder column. Rinse the flask with 0.5-1.0 mL hexane. Remove the concentrator tube. Place the tube on the tube heater set at 40 degrees C. Blow ultra-pure

nitrogen across the surface of the solvent layer in the tube. Rinse the sidewalls of the tube periodically while concentrating. Bring the extract down to a volume of 1.0 mL. Quantitatively transfer the solution from the concentrator tube to a 5 mL volumetric flask and bring to a final volume of 5.0 mL with hexane.

Analyze the BNA verification by GC/MS. If the blank exceeds one half of the CRQL of any analyte, pump additional methylene chloride through the GPC system for 1-2 hours. Analyze another GPC BNA verification blank to ensure the system is sufficiently clean. Repeat the methylene chloride pumping if necessary.

Analyze the pesticide and Aroclor verifications by GC. The recoveries of each of the single component analytes must be 80 to 110 percent. The Aroclor pattern should be the same as with previously run standards. If recoveries are out of the acceptance window or if changes in the relative peak heights of the patterns of the Aroclor are observed, the column must be recalibrated.

SAMPLE EXTRACT CLEANUP:

In order to prevent overloading of the GPC column, highly viscous samples must be diluted prior to cleanup and loaded into several loops.

Flow rate should be check every other day that samples are run. Verify the flow rate as described in Calibration of the GPC Column section.

Fill the solvent and rinse reservoirs with methylene chloride. Load filtered samples into labeled sample tubes and then onto the injection ports. Place collect lines into assembled and labeled 300 mL K-D flasks.

Instrument Programming:

Sample Load Time

Press INDEX to read,"RUN SAMPLE LOAD AUTOTIMER (NO=0)-?". Press ENTER. Load sample port #1 with 10 mL methylene chloride. Press INDEX to read, "ENTER, TO START/STOP TIMER 0:00.0?". Press ENTER. The timer will start, and the sample will be injected. Press ENTER again to stop the timer as the sample reaches the bottom of the tube. Press INDEX to read, "LOAD TIME = *:**.* SAVE TO PROGRAM = *?". Enter a program number, which can be 1 through 5, and press ENTER. Press INDEX to continue.

Setting Program Parameters

Press INDEX to read, "CURRENT PROGRAM = *?". Enter the desired program number followed by ENTER. Press INDEX.

When the message, "SAMPLE LOAD TIME = *:**.*?" is displayed, press INDEX to accept.

Press INDEX to read, "DUMP TIME = **:**?". Enter the dump time. Press ENTER.

Press INDEX to read, "COLLECT TIME = **:**?". Enter the collect time. Press ENTER.

Press INDEX to read, "WASH TIME = **:**?". Enter 10:00. Press ENTER.

Press PROGRAM SAVE to read "SAVE TO PROGRAM = *?". Enter a program number and press ENTER. The program must be saved following any changes. Up to five programs, number 1 through 5, can be saved.

Sample Cleanup

Press INDEX to read, "START WITH SAMPLE = **?". Enter the starting sample port number. Press ENTER.

Press INDEX to read, "TERMINAL SAMPLE = **?". Enter the last sample port number. Press ENTER.

Press INDEX to read, "PROCESS WITH MULTI PROGRAMS (NO=0)-?". Enter 0 = NO or 1 = YES depending if one desires semi-volatile or pesticide cleanup or a multi program for both semi-volatile and pesticide cleanup. Refer to the OPERATION MANUAL for information on multiprogramming.

Press INDEX to read, "LAST SAMPLE = ** TIME = **:** PUSH RUN ?". Push RUN to start GPC sequence.

FINAL CONCENTRATION OF EXTRACT:

When the GPC displays, "SAMPLE ** PROCESSING COMPLETE", cleanup is finished. Attach a three-ball Snyder column to the top of the Kuderna Danish flask.

Continue the concentration as follows:

Semi-Volatiles: Place the flask on the hot (70-75 degrees C) water bath. Gently swirl the flask until boiling occurs. Concentrate to an apparent volume of 1-2 mL. Remove the flask from the water bath. Allow to drain and cool for 15 minutes. Remove the three-ball Snyder column. Rinse the flask with 0.5-1.0 mL methylene chloride. Remove the concentrator tube. Place the tube on the tube heater set at 40 degrees C. Blow ultra-pure nitrogen across the surface of the solvent layer in the tube. Rinse the sidewalls of the tube periodically while concentrating. Bring the extract down to a volume of 500 uL. Transfer 400 uL to an injection vial with a clean syringe. Cap with a crimp cap.

Pesticides: Place the flask on the hot (80-90 degrees C) water bath. Gently swirl the flask until boiling occurs. Concentrate to approximately 20 mL. Perform a solvent exchange by adding 50 mL hexane to the top of the three-ball Snyder column. Concentrate to an apparent volume of 1-2 mL. Remove the flask from the water bath. Allow to drain and cool for 15 minutes. Remove the three-ball Snyder column. Rinse the flask with 0.5-1.0 mL hexane. Remove the concentrator tube. Bring the extract to a final volume of 5 mL with hexane. Transfer to a 12 mL amber vial. Cap with a teflon-lined screw cap. Pesticide extracts may require further cleanup depending on analytical protocol. Proceed to RMT Standard Operating Procedure 3-SVO-25 for Florisil cartridge cleanup if necessary.